Breeding for functional foods

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Introduction

A large number of oilseeds with genetically modified crop production traits have been developed in recent years. Examples include herbicide resistance, insect resistance, disease resistance and male sterility and restoration (Green and Salisbury 1998). Gene technology also provides substantially increased opportunities to alter the composition of grains to better match current and emerging food and industrial uses. In particular, there is a major focus on the development of oilseed products as natural health products and functional foods.

Natural health products

Natural health products are used for treatment, mitigation or prevention of disease and/or maintaining or promoting health. These products include supplements like vitamins and essential fatty acids as well as nutraceuticals. Nutraceuticals are products isolated or purified from foods and generally sold in medicinal forms not associated with food. They are sold encapsulated or in bottles.

Alpha-linolenic acid (omega-3)

Alpha-linolenic acid is effective in lowering blood cholesterol levels, reducing clotting of blood platelets (reducing risk of stroke) and lowering blood pressure. There is the potential to increase levels of alpha-linolenic acid in linseed and other oilseeds, both through conventional breeding and biotechnology.

Gamma-linolenic acid

Gamma-linolenic acid is effective in reducing symptoms of rheumatoid arthritis, treating diabetic neuropathy and reducing symptoms of skin disorders, including atopic eczema and dermatitis. It is currently produced from evening primrose or borage. The Δ6-desaturase gene has been transferred into canola resulting in higher yields of gamma-linolenic acid than in borage and evening primrose.

Nutraceuticals

Biotechnology is being used to develop a range of nutraceutical products in oilseeds. Examples include flu and rabies vaccines (addition of genes for viral proteins) and pharmaceutical peptides such as hirudin (a blood anti-coagulant protein produced by a gene from leeches) and thrombin. The oleosin proteins that encase the oil storage bodies in the seed have been successfully engineered to carry these high value pharmaceutical peptides (van Rooijen and Moloney 1995).

Functional foods

Functional foods are foods that demonstrate physiological benefits and/or reduce risk of chronic diseases beyond the basic nutritional function.

High oleic, low linolenic acid

One breeding objective has been to develop healthy, high-stability cooking oils that can be used directly in the food service sector without the need for hydrogenation. To ensure stability during long-life cooking applications, oils must have relatively low levels of polyunsaturated fatty acids. In particular, linolenic acid must be very low as it is rapidly oxidised to give undesirable off-flavours. Currently, high-stability vegetable oils are obtained either by using imported palm oils that have naturally high stability, or by partially hydrogenating polyunsaturated oils (cottonseed, canola, soybean) to convert polyunsaturates back to monounsaturates and saturates. Both approaches are nutritionally undesirable, palm oils because they contain high levels of cholesterol-raising saturates, and hydrogenation because it results in production of cholesterol-raising *trans* fatty acids. It would be preferable to modify the fatty acid composition of locally-grown oilseeds to have the required nutritional and functional properties. This has now been achieved by the development of high-oleic forms of all the major oilseed crops, through either conventional breeding or through the inactivation of the Δ12-desaturase gene using gene silencing techniques. Silencing of Δ12-desaturase has been used to raise oleic acid levels to 89% and 75% in canola oil from *Brassica napus* and *B. juncea* respectively (Stoutjesdijk *et al.* 2000), and to 77% in cottonseed oil (Liu *et al.* 2000). Similar approaches have been used to develop soybean oils with 88% oleic acid.

High stearic acid

As well as being used in liquid cooking applications, vegetable oils are also hydrogenated to produce solid fats for use in margarines and shortenings. In this case, hydrogenation is used to increase the level of high melting-point saturates and *trans* fatty acids. Stearic acid (C18:0) is a high melting point saturate that is known to be neutral with respect to blood cholesterol levels, however it is only a very minor component in the main seed oils. The development of oilseeds with naturally high levels of stearic acid should provide oils having melting points high enough for their direct use in solid fat applications without the need for hydrogenation. Oils with up to 40% stearic acid have now been developed in canola (Knutzon *et al.* 1992) and in cottonseed (Liu *et al.* 2000) by using gene technology to silence the Δ9-desaturase gene in the seed. The use of such high-stearic oils instead of hydrogenated oils as the hardstock in margarines could have positive nutritional effects through replacement of cholesterol-raising *trans* fatty acids with neutral stearic acid.

Alpha-tocopherols (vitamin E)

Tocopherols are essential nutrients and natural anti-oxidants. They are beneficial in the treatment of atherosclerotic disease. Increases in the levels of highly effective antioxidants in food oils would be a valuable addition to the human diet as well as providing enhanced stability to the oils during processing. Biotechnology has been used to increase levels of alpha-tocopherols in *Arabidopsis*.

Phytosterols

Dietary cholesterol is readily absorbed into the bloodstream. Phytosterols (for example, brassicasterol, stigmasterol) are not absorbed and they reduce the absorption of dietary cholesterol. In clinical trials, margarines with 8% phytosterols were able to significantly reduce blood cholesterol levels. These phytosterol-containing margarines have recently been introduced onto the Australian market under the Logicol® and ProActiv® brands. Phytosterols are currently obtained as by-products of pine wood pulping or sunflower oil distillates. The possibility exists to use gene technology to greatly increase the naturally low levels of phytosterols in oils up to levels that have a nutritional benefit. Canola and soybean oils with 2–5% phytosterols have recently been produced by over-expressing the rate limiting enzyme in sterol synthesis.

Beta-carotene (Golden mustard)

Approximately 250 million people worldwide suffer from vitamin A deficiency. Vitamin A deficiency causes vision impairment, inability to absorb proteins and nutrients and reduced immune function. One of the highest profile applications of gene technology in human nutrition has been the recent development of rice containing β-carotene, the compound that the body converts into vitamin A. This product has the potential to alleviate vitamin A deficiency in many parts of the developing world. It was achieved by the introduction of genes for the three key enzymes in the β-carotene pathway, namely phytoene synthase, carotene desaturase and lycopene β-cyclase (Ye *et al.* 2000).

Seed oils also contain low levels of β-carotene and research has shown that β-carotene levels in canola seed can be raised up to 50-fold by the introduction of the phytoene desaturase gene targeted to the plastid (Shewmaker *et al.* 1999). As β-carotene is lipid-soluble, the majority of it will be extracted in the seed oil. Golden mustard (*B. juncea*) oil high in β-carotene is now being developed using biotechnology, especially focussed at India (James 2001).

Long chain polyunsaturated fatty acids ('Fish oils')

Long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (C20:4ω6, AA), eicosapentanoic acid (C20:5ω3, EPA) and docosahexanoic acid (C22:6ω3, DHA), have been demonstrated to have important roles in brain and retinal development, and as precursors for synthesis of various prostaglandins regulating important bodily functions, such as anti-inflammatory reactions and blood platelet aggregation. Although the human body can produce LC-PUFA by elongation and desaturation of dietary linoleic acid (C18:2) and α-linolenic acid (C18:3), it does so inefficiently. It is therefore important to have an adequate dietary intake of these fatty acids, with marine oils a rich source of EPA and DHA. However, concerns about the ability of global fish stocks to meet this requirement in the long term have lead to interest in developing oilseeds that naturally contain moderate to high levels of LC-PUFA.

The LC-PUFA fatty acids present in fish oils are the result of accumulation of ingested fatty acids originating from microalgae. A number of research groups have now cloned elongase and desaturase genes responsible for LC-PUFA synthesis from micro-organisms, and these genes are being introduced into oilseeds in order to assemble the pathway for synthesis of LC-PUFA from linoleic acid and α-linolenic acid (Parker-Barnes *et al.* 2000). Recently, the accumulation of up to 17% stearidonic acid (C18:4ω3, SDA) has been achieved in rapeseed by the introduction of genes for Δ6-, Δ12- and Δ15-desaturases (Ursin *et al.* 2000). Furthermore nutritional studies with SDA indicate that it is efficiently converted to EPA and DHA by the human body, suggesting that it may be valuable to produce seed oils rich in SDA, as well as assembling the full EPA/DHA pathway in oilseeds. Thus it is highly likely that genetically modified oilseeds having nutritionally effective levels of LC-PUFA will soon be a reality.

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